REMARKS

I. Status of the Claims

Claims 1-20, 22-39, 41, and 44-55 were pending at the time of mailing of the final Office Action. Claims 3-8, 11-20, 33-38 and 53-55 are presently withdrawn from consideration as being directed to a non-elected invention. Claim 1 been amended in the Amendment set forth herein. No claims are canceled. New claim 56 has been added. New claim 56 is a member of the elected Group I invention. Support for the amendments to the claims is discussed in greater detail below. Support for new claim 1 can be found generally throughout the specification, such as in the claims as originally filed and in FIG. 56. Claims 1-20, 22-39, 41, and 44-56 are presently under consideration.

II. The Written Description Rejections are Overcome

Claims 1, 2, 9, 10, 22-32, 39, 41, and 44-52 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. The Examiner asserts that the claims lack support for the chemical structure depicted in claim 1, and that Applicants' previous arguments do not support that the replacement of a methyl group for a hydrogen was a typographical error. Applicants respectfully traverse.

The Federal Circuit has stated that the test for the written description requirement is "whether the application relied upon 'reasonably conveys to the artisan that the inventor had possession at the time of the later claimed subject matter.'" *In re Daniels*, 144 F.3d 1452, 1456, 46 USPQ2d 1788, 1790 (Fed. Cir. 1998). See also *Markman v. Westview Instruments, Inc.* 52 F.3d 967, 34 USPQ 2d 1321 (Fed. Cir. 1995) (*en banc*) ("Claims must be read in view of the specification, of which they are a part."). Applicants stand by their previous arguments for support, including the correctly recited structures shown in figures 39, 40, and 41. One of

ordinary skill in the field, in view of the information in the specification as a whole including the numerous compounds recited in the figures and working examples, would have understood that Applicants were in possession of the structure set forth in claim 1, and that the replacement of the methyl group for a hydrogen was an error.

Further, the Examiner is directed to *MPEP* §2163, Guidelines for the Examination of Patent Applications which recites "[a]n amendment to correct an obvious error does not constitute new matter where one skilled in the art would not only recognize the existence of the error in the specification, but also recognize the appropriate correction." *Id.* citing *In re Oda*, 443 F.2d 1200, 170 USPQ 268 (CCPA 1971) (reversing a rejection based on new matter where the claims were corrected to recite "nitric" instead of "nitrous" and noting that "one skilled in the art would appreciate not only the existence of error in the specification but what the error is. As a corollary, it follows that when the nature of this error is known it is also known how to correct it. We therefore disagree with the board's first conclusion that the change of 'nitrous' to 'nitric' is 'new matter.'")

The burden is on the Examiner to set forth a *prima facie* case of unpatentability. See *In re Alton*, 76 F.3d 1168, 1175, 37 USPQ2d 1578, 1581 (Fed. Cir. 1996). Here, the Examiner has not set forth any evidence to indicate that one skilled in the art would not have recognized a description of the compounds covered by currently pending claim 1. The instant specification recites working examples setting forth detailed methods concerning the isolation of the specific (correct) compounds set forth in the figures from *Acacia sp.*(*see, e.g.,* Example 2, p. 149, line 4 – page 172, line 3). The Examiner seems to have disregarded this information.

The Examiner is also directed to *Ex Parte Marsili*, 214 USPQ 904 (Bd. Pat. App. & Interfs. 1979), a 1979 decision from the Board of Patent Appeals and Interferences which was recently cited by the Court of Appeals for the Federal Circuit in *Chen v. Bouchard*, 347 F.3d

1299, 68 USPQ2d 1705 (Fed. Cir. 2003). In *Ex Parte Marsili*, the applicants initially disclosed that they had prepared compounds containing an imidazoline (also called a "dihydro-imidazole") ring, when in fact the described methods produced compounds having more stable, aromatic imidazole rings. In reversing a new matter rejection raised after the applicant sought to correct the structure in claim 1 of the patent application, the Board found that the question was not one of "adding characteristics not previously mentioned," but rather of "changing the original description of a product which . . . was described by sufficient characteristics to distinguish it." *Id.* at 905. The Board accordingly found that "the products described, exemplified and claimed by Appellants inherently had and have now the structure given in the amendment in question," and held that "the changes made in this amendment do not constitute new matter." *Id.* at 906. In *Chen v. Bouchard*, the Federal Circuit distinguished the facts (which concerned an allegation of new matter where the applicant attempted to amend the claims to recite certain chemical derivatives not recited in the original application) of that case from *Ex Parte Marsili*, noting that "[t]he '261 and '423 applications, in contrast, not only do not disclose the structural formulae of any cyclopropataxol derivatives, they apparently also do not disclose any analytical data or other characteristics of such derivatives." *Chen*, 347 F.3d at 1306. *Ex Parte Marsili* is on point to the instant facts, as Applicants have provided data and detailed information concerning identification and isolation of the claimed compounds from a natural substrate, including numerous specific examples in the figures. Thus, the compounds described and exemplified in the specification inherently had and now have the methyl group at issue in pending claim 1.

In view of the foregoing, it is respectfully requested that the written description rejections under 35 U.S.C. §112, first paragraph, be withdrawn.

III. The Indefiniteness Rejections Are Overcome

Claims 1, 2, 9, 10, 22-32, 39, 41, and 44-52 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. In particular, claim 1 has been objected to for reciting the phrase "further defined." Applicants respectfully traverse.

Without conceding that the claims as originally written were indefinite, Applicants note that the claims have been amended to no longer recite "further defined. In view of the foregoing, Applicants respectfully request that the rejection of the claims as being indefinite under 35 U.S.C. §112, second paragraph, be withdrawn.

IV. The Obviousness Rejections Are Overcome

1. Rejection of Claims 1, 2, 9, 10, 24-28, 31-32, 41, and 44-52 Based on Arntzen-1 in View of Ni

Claims 1, 2, 9, 10, 24-28, 31-32, 41, and 44-52 are rejected under 35 U.S.C. §103(a) as being unpatentable over Arntzen *et al.* (U.S. Patent 6,444,233; hereinafter "Arntzen-1") and further in view of Ni *et al.* (U.S. Patent 5,965,421; hereinafter "Ni"). Applicants respectfully traverse.

a) The Examiner's Argument in Support of Obviousness is Deficient

The Examiner admits that Arntzen-1 does not provide any teaching concerning inflammatory bowel disease. The Examiner cites to col. 16, lines 5-12 of Ni as teaching that dysregulation of NF-κB activation has been linked to rheumatoid arthritis or inflammatory bowel disease. Dysregulation is a generic term, and may mean increased or decreased NF-κB activation in the context of this statement. Applicants have attached as Exhibit 1 a definition of "dysregulation" from the Merriam-Webster online Medical Dictionary, which provides that

dysregulation is defined as "impairment of a physiological regulatory mechanism (as that governing metabolism, immune response, or organ failure)." Thus, one of ordinary skill in the art would not have any reasonable expectation of success that inhibitors of NF-κB activation would decrease NF-κB activation a subject with rheumatoid arthritis or inflammatory bowel disease. The mere mention of rheumatoid arthritis and inflammatory bowel disease in Ni is not sufficient to establish a *prima facie* case of obviousness. Neither Arntzen-1 nor Ni provides any specific information that would provide any reasonable expectation of success that administration of the monoterpene-containing compounds of the present invention would reduce NF-κB activation in a subject with rheumatoid arthritis or inflammatory bowel disease.

New claim 56, which depends from claim 1, is nonobvious for the foregoing reasons.

b) Declaration of Dr. Anderson Setting Forth Supplemental Data Supporting Therapeutic Efficacy

As further evidence of nonobviousness of the claimed invention (particularly new claim 56), attached as Exhibit 2 is a Declaration of Dr. Roger Anderson (hereinafter "the Declaration") which provides unexpected efficacy of a compound falling within the scope of the instant claims in treating inflammatory bowel disease. A study protocol to determine the efficacy of Avicin D in treating dextran sulfate sodium (DSS) -induced inflammatory bowel disease in mice is discussed in the Declaration, and a summary is set forth as Exhibit A of the Declaration.

The study protocol is discussed in ¶5 of the Declaration. Briefly, fifty male mice aged 7 weeks were acclimated for one week at the laboratory facility, and then divided by weight into 5 groups. Group 1, the control group, received subcutaneous vehicle only on a daily basis from days 1-51. Group 2 received a subcutaneous dose of glatiramer acetate (100 mg/kg) daily on days 1-51. Glatiramer acetate is an agent that is known to suppress inflammatory bowel disease in some animal models. Mice in groups 3 and 4 received a subcutaneous dose of Avicin D (0.25

and 0.5 mg/kg, respectively) daily on days 1-51. Group 5 mice were dosed with an escalating dose of Avicin D, as shown in the treatment protocol. See Exhibit A, section 4.1 and 4.2. All mice in group 5 were euthanized for cause on day 36. Body weight and cage side observations were performed daily. Clinical observations and Disease Activity Index (DAI) scores were assessed approximately every 2 days. At study termination, mice were sacrified and colons were harvested, flushed, weighed, and lengths recorded. Colons were fixed, stored, and sent for histology analysis.

Following study termination, post-flush colon weights were found to be significantly different in all groups. Declaration, ¶7, citing to Exhibit A, section 5.6. The vehicle group had significantly more weight as compared to the high dose Avicin group ($p<0.05$ two-tailed t test). Colon weight/length ratio has been reported to be an indicator of colitis progression. The ratio was significantly higher in the vehicle group as compared to the Avicin D high dose group (two tailed t test).

Formalin-fixed sections were examined for histology as discussed in ¶8 of the Declaration. Disease severity scores were assigned using the parameters set forth in section 5.7 of Exhibit A. Group 4 (high dose Avicin D group) showed significantly lower medial and distal disease severity as compared to the vehicle group, the low-dose Avicin D group (Group 3) and the positive control group (Group 2). Total average scores were calculated and are set forth in section 5.8 of Exhibit A. Histological analysis showed that disease was induced in all groups. Positive control Group 2 did not show any significant improvement in disease outcome. Group 4 (0.5 mg/kg Avicin D) showed significantly lower number of severe lesions as compared to all other three groups. Group 4 showed a significantly lower total severity score than Group 1, 2, or 3 ($p<0.05$ two tailed test, one way ANOVA), thus evidencing therapeutic efficacy. See Exhibit A, section 5.8.

In view of the foregoing, it is respectfully requested that claims 1, 2, 9, 10, 24-28, 31-32, 41, and 44-52 are not unpatentable under 35 U.S.C. §103(a) based on Arntzen-1 in view of Ni.

2. Rejection of Claims 1, 2, 9, 10, 24-28, 31-32, 41, and 44-52 Based on Arntzen-2 in View of Ni

Claims 1, 2, 9, 10, 24-28, 31-32, 41, and 44-52 are rejected under 35 U.S.C. §103(a) as being unpatentable over Arntzen *et al.* (WO 1999/59578; hereinafter "Arntzen-2") and further in view of Ni (as above). Applicants respectfully traverse.

The Examiner has admitted that Arntzen-2 does not provide any information concerning rheumatoid arthritis or inflammatory bowel disease. As with the previous rejection, the Examiner relies on Ni for providing that dysregulation of NF-κB activation has been linked to rheumatoid arthritis or inflammatory bowel disease. However, for the reasons discussed above (the discussion of which is incorporated into this section), one of ordinary skill in the art would not have a reasonable expectation of success that the monoterpene-containing compounds as set forth in the claims would reduce NF-κB activation in a subject with rheumatoid arthritis or inflammatory bowel disease. Applicants further cite to the supplemental data in the declaration of Dr. Anderson discussed above.

In view of the foregoing, it is respectfully requested that claims 1, 2, 9, 10, 24-28, 31-32, 41, and 44-52 are not unpatentable under 35 U.S.C. §103(a) based on Arntzen-2 in view of Ni.

3. Rejection of Claims 22-23 and 29-30 Based on Arntzen-1 in View of Ni

Claims 22-23 and 29-30 are rejected under 35 U.S.C. §103(a) as being unpatentable over Arntzen-2 (U.S. 6,444,233), and further in view of Ni. Applicants respectfully traverse.

Arntzen-2 appears to be the U.S. counterpart of Arntzen-1. For the reasons previously set forth concerning Artnzen-1 and Ni, one of ordinary skill in the art would not have a reasonable expectation of success that the monoterpene-containing compounds as set forth in the claims

would reduce NF-κB activation in a subject with rheumatoid arthritis or inflammatory bowel disease.

The claims at issue recite particular isomers. Applicants note that homology and isomerism involve close structural similarity which must be considered with all other relevant facts in determining the issue of obviousness. *MPEP* §2144.09, citing *In re Mills*, 281 F.2d 218, 126 USPQ 513 (CCPA 1960); *In re Wiechert*, 370 F.2d 927, 152 USPQ 247 (CCPA 1967). Further, as set forth in MPEP §2144.09, homology should not be automatically equated with *prima facie* obviousness because the claimed invention and the prior art must each be viewed "as a whole." *In re Langer*, 465 F.2d 896, 175 USPQ 169 (CCPA 1972). The Examiner must consider the teachings of both references. When so done, it is clear that the combination of references provides no reasonable expectation of success that the isomers set forth in the claims can find application in downregulating NF-κB activation in a subject with rheumatoid arthritis or inflammatory bowel disease.

In view of the foregoing, it is respectfully requested that claims 22-23 and 29-30 are not unpatentable under 35 U.S.C. §103(a) based on Arntzen-1 in view of Ni.

4. Rejection of Claim 39 Based on Arntzen-2 in View of Ni

Claims 39 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Arntzen-2 and further in view of Ni. Claim 39 recites a concentration range. Applicants respectfully traverse.

There is no *prima facie* case of obviousness for the reasons discussed above, the discussion of which is incorporated into this section. One of ordinary skill in the art would not have a reasonable expectation of success that the monoterpene-containing compounds as set forth in the claims would reduce NF-κB activation in a subject with rheumatoid arthritis or inflammatory bowel disease.

In view of the foregoing, it is respectfully requested that claim 39 is not unpatentable under 35 U.S.C. §103(a) based on Arntzen-1 in view of Ni.

IV. The Nonstatutory Obviousness-Type Double Patenting Rejection Is Overcome

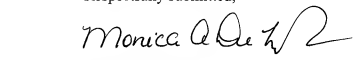
Claims 1, 2, 9, 10, 21-32, 39-43, 46 and 48-51 are rejected on the grounds of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-9 and 16-21 of U.S. Patent 6,962,720. The Examiner contends that while the conflicting claims are not identical, they are not patentably distinct from each other because the '720 patent and the rejected claims both recite methods of treating inflammation by administering a monoterpene.

Applicants respectfully traverse. As noted above, the present claims are drawn towards inhibiting inflammation in a subject with inflammatory bowel disease. Inflammatory bowel disease is not mentioned in the '720 patent. The Examiner has provided no factual evidence to support an obviousness rejection of the amended claims. *See* MPEP § 2142 ("The examiner bears the initial burden of factually supporting any *prima facie* case of obviousness."). There are many different types and causes of inflammation. Nothing in this reference teaches or suggests treating subjects with inflammatory bowel disease. Nor has the Examiner provided any evidence that treatment of inflammatory diseases are identical, and that all such diseases would be expected to respond in a similar manner to anti-inflammatory treatment. The Examiner has failed to establish that one of ordinary skill in the art would have a reasonable expectation of success for treatment of inflammatory bowel disease. Thus, no *prima facie* case of obviousness has been established. Therefore, Applicants respectfully request that this rejection bc withdrawn.

V. Conclusion

This is a full and complete response to the Office Action mailed February 21, 2008. In view of the foregoing, it is respectfully submitted that each of the pending claims is in condition for allowance, and a Notice of Allowance is earnestly solicited. The Examiner is invited to contact the undersigned attorney at (512) 536-5605 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



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Date: December 14, 2009

dysregulation

One entry found.

Main Entry: **dys·reg·u·la·tion**
Pronunciation: \ˌdis-ˌreg-yə-ˈlā-shən, -ˌreg-ə-\
Function: *noun*
: impairment of a physiological regulatory mechanism (as that governing metabolism, immune response, or organ function)
—**dys·reg·u·lat·ed** \-ˈreg-yə-ˌlāt-əd, -ˈreg-ə-\ *adjective*

Pronunciation Symbols

EXHIBIT 1

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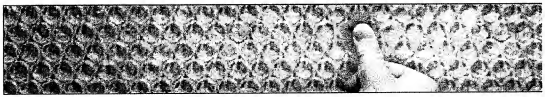
dysregulation



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Jordan U. Gutterman, *et al.*

Serial No.: 09/992,556

Filed: November 16, 2001

For: INHIBITION OF NF-κβ BY TRITERPENE COMPOSITIONS

Confirmation No. 5224

Group Art Unit: 1612

Examiner: Webb, Walter E.

Atty. Dkt. No.: CLFR:009US

CERTIFICATE OF ELECTRONIC TRANSMISSION
37 C.F.R. § 1.8

I hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office via EFS-Web on the date below:

12/14/09 — Monica A. De La Paz
Date

DECLARATION OF ROGER S. ANDERSON

I, Roger S. Anderson, hereby declare as follows:

1. I am a citizen of the United States residing at 3378 Point White Drive, Bainbridge Island, Washington, 98110.

2. I am employed as Chief Technology Officer (CTO) of Qwell Pharmaceuticals, Seattle, Washington.

3. Qwell Pharmaceuticals has licensed the technology that is the subject of the above-referenced patent application.

4. Qwell Pharmaceuticals contracted with The Jackson Laboratory-West (West Sacramento, California) for The Jackson Laboratory – West to conduct a study to determine the

efficacy of Avicin D in treating dextran sulfate sodium (DSS) -induced inflammatory bowel disease in mice. The treatments were given in prophylactic and therapeutic modes. The results of this animal study have relevance for the treatment of inflammatory bowel syndrome in humans and demonstrate effectiveness of the claimed methods.

5. A copy of the study protocol is set forth as Exhibit A. Briefly, fifty male mice aged 7 weeks were acclimated for one week at the laboratory facility, and then divided by weight into 5 groups. Group 1, the control group, received subcutaneous vehicle only on a daily basis from days 1-51. Group 2 received a subcutaneous dose of glatiramer acetate (100 mg/kg) daily on days 1-51. Glatiramer acetate is an agent that is known to suppress inflammatory bowel disease in some animal models. Mice in groups 3 and 4 received a subcutaneous dose of Avicin D (0.25 and 0.5 mg/kg, respectively) daily on days 1-51. Group 5 mice were dosed with an escalating dose of Avicin D, as shown in the treatment protocol. See Exhibit A, section 4.1 and 4.2. All mice in group 5 were euthanized for cause on day 36. Body weight and cage side observations were performed daily. Clinical observations and Disease Activity Index (DAI) scores were assessed approximately every 2 days. At study termination, mice were sacrified and colons were harvested, flushed, weighed, and lengths recorded. Colons were fixed, stored, and sent for histology analysis.

6. DAI scoring represents non-invasive observation of disease severity. Factors examined included prolapse, stool consistency, and fecal occult blood. Most animals in the vehicle group shoed diarrhea. Average DAI was calculated for all groups and found to be higher

in the vehicle group. DAI representing stool consistency was higher in the vehicle group during the 4th DSS cycle. Cumulative scores were calculated for each week of the study and showed disease induction in all groups, with greatest severity in the vehicle group. Exhibit A, section 5.5. All of the groups showed an increase in disease activity during the fourth week of observation. Disease activity scores continued to be higher during the fifth week in all groups except for the Avicin D group 4 (0.5 mg/kg). This group showed less severity and disease induction as compared to other groups.

7. Following study termination, post-flush colon weights were found to be significantly different in all groups. See Exhibit A, section 5.6. The vehicle group had significantly more weight as compared to the high dose Avicin group ($p<0.05$ two-tailed t test). Colon weight/length ratio has been reported to be an indicator of colitis progression. The ratio was significantly higher in the vehicle group as compared to the Avicin D high dose group (two tailed t test).

8. Formalin-fixed sections were examined for histology. Disease severity scores were assigned using the parameters set forth in section 5.7 of Exhibit A. Histology findings and gradings of individual slides was performed without knowledge of treatment (blinded). Group 4 (high dose Avicin D group) showed significantly lower medial and distal disease severity as compared to the vehicle group, the low-dose Avicin D group (Group 3) and the positive control group (Group 2). Total average scores were calculated and are set forth in section 5.8 of Exhibit A. Histological analysis showed that disease was induced in all groups. Positive control Group 2 did not show any significant

improvement in disease outcome. Group 4 (0.5 mg/kg Avicin D) showed significantly lower number of severe lesions as compared to all other three groups. Group 4 showed a significantly lower total severity score than Group 1, 2, or 3 ($p<0.05$ two tailed test, one way ANOVA). See Exhibit A, section 5.8.

9. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DEC 11, 2009

Date

Roger S. Anderson

3 MICE AND HOUSING

A total of 50 male C57BL/6J mice aged 7 weeks were received from The Jackson Laboratory Sacramento facility on December 1, 2008. The mice were ear notched for identification using the standard mouse ID format (IVS16741001 to IVS16741050). The first eight characters of the ID format represented the project number (IVS16741). The final three digits of the ID format represented the mouse number. Mice were housed at a density of 5 per cage on bedding containing natural flora to ensure the transfer of enteric flora required to accurately model colitis. Bed-o'cobs® corn cob bedding was provided and was changed every two weeks or as needed. The animal room was lighted entirely with artificial fluorescent lighting on controlled 12 hr light/dark cycle (6 a.m. to 6 p.m. light). The normal temperature and relative humidity ranges in the animal rooms were maintained at 22 ±4°C and 50 ±15%, respectively. The animal room was set for 15 air exchanges per hour. Filtered tap water acidified to a pH of 2.8 to 3.2 was provided *ad libitum*. LabDiet 5LL4 was provided *ad libitum*.

4 METHODS AND RECORDS

After a one week acclimation, mice were divided by weight into 5 groups (n=10).

Beginning on Day 1:

a. Daily dosing (SC) in Groups 3, 4, and 5 (doses of Avicin D) (Days 1-51)
b. Daily dosing (SC) in Group 2 with GA (Days 1-51)

Beginning on Day 9:

a. Mice received first cycle of 1.5% DSS in acidified water ad libitum for 5 days followed by 7 days of water alone, followed by a repeat of 2 cycles of 1.5% DSS in acidified water for 5 days followed by 5 days of water alone. A fourth cycle of DSS (2.5%) was repeated for 5 days followed by 4 days of water alone.
b. DSS 1st cycle: days 9-13
c. DSS 2nd cycle: days 21-25
d. DSS 3rd cycle: days 31-35
e. DSS 4th cycle: days 42-47

Body weight was recorded daily. Cage side observations were conducted weekly. Clinical observations and Disease Activity Index (DAI) scores were assessed approximately every 2 days.

Mice in group 5 were dosed with escalating dose of Avicin D, as shown in treatment protocol. All mice from this group were euthanized for cause on day 36.

Blood was taken and processed to plasma, prior to dosing and at study termination.

EXHIBIT A

At study termination, mice were sacrificed and colons were harvested, flushed, weighed, and lengths recorded. Colons were fixed and stored, until they were sent for histology analysis.

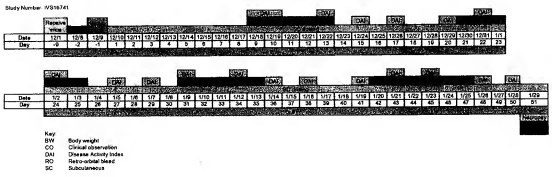
Blood Processing Details:

Blood Collected on 12/08/08 and 01/29/09: Blood was processed for plasma by collection into an EDTA tube and spun for 12 minutes at 12,000 RPMs in a 4°C centrifuge, and then transferred to 0.5 mL microcentrifuge tubes for storage at -80°C until sent for analysis.

4.1 Treatment Protocol

Gp	n	Treatment	Volume (mL/kg)	Dose (mg/kg)	Route	Dose Regimen
1	10	Vehicle	n/a	n/a	SC	Daily, days 1-51
2	10	Glatiramer Acetate	10	100	SC	Daily, days 1-51
3	10	Avicin	10	0.25	SC	Daily, says 1-51
4	10	Avicin	10	0.5	SC	Daily, days 1-51
5	10	Avicin	10	0.5 0.75 n/a 0.75 1.0 1.5 2.0	SC	Daily, days 1-2 Daily, days 3-4 No dosing, days 5-9 Daily, days 10-20 Daily, days 21-24 Daily, days 25-27 Daily, days 28-35

4.2 Procedural Timeline



5 RESULTS

The study log and all related information have been archived at The Jackson Laboratory-West at 960 Riverside Parkway, West Sacramento, CA 95605. The results and raw data are embedded in Appendix 1.

5.1 Observations

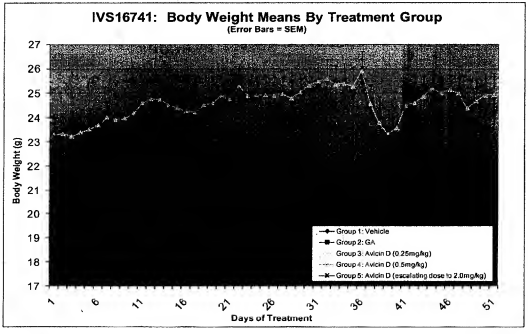
All mice received daily cage-side observation, and the following notes indicate physical and behavioral developments. All mice tolerated the treatments and all weekly clinical observations report bright, alert, responsive and hydrated mice, except as noted below:

Observation Notes:

- On 01/13/09, Mouse IVS16741049 (Group 5), was found dead.
- On 01/14/09, Mice IVS16741039 and IVS16741050 (Group 5), were found dead. Mice IVS16741036, IVS16741037, IVS16741038, IVS16741040, IVS16741046, IVS16741047, and IVS16741048 were euthanized for cause.
- On 01/19/09, Mice IVS16741007 (Group 1), and IVS16741019 (Group 2), were found dead.
- On 01/29/09, Mouse IVS16741034 (Group 2), was found dead prior to scheduled necropsy.

5.2 Body Weight:

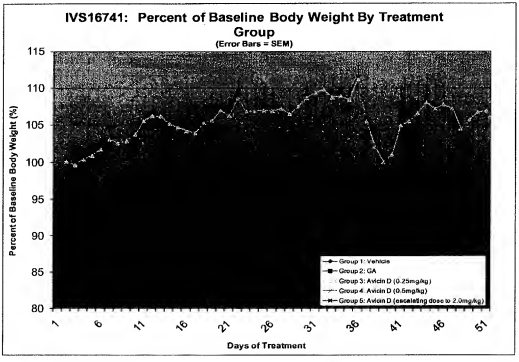
Body weights (g) were averaged by treatment group. Error bars represent standard error of the mean (SEM).



5.3 Change in Body Weight:

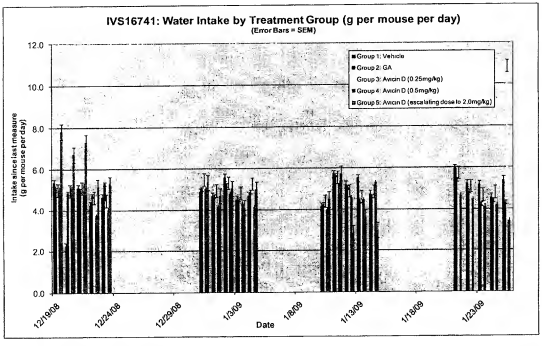
Body weight change was calculated for each mouse by subtracting the body weight on the first day of dosing (baseline) from the body weight on the last day of dosing (final) and calculating the percent change from baseline body weight: [=100*(FinalWeight –

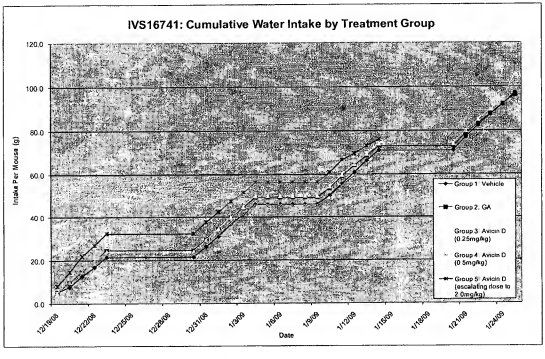
BaselineWeight)/BaselineWeight]. Average percent body weight change was then calculated by treatment group. Error bars represent standard error of the mean (SEM).



5.4 Water Intake:

Intake per mouse per day was calculated based on water intake per cage. Mean intake by treatment group was then calculated weighted by number of mice per cage. Therefore, measurements from cages with 5 mice per cage receive 5/8 of weighting and cages with 3 mice per cage receive 3/8 weighting when 8 mice per treatment group, etc. Error bars represent standard error of the mean (SEM).


IVS16741: Water Intake by Treatment Group (g per mouse per day)
(Error Bars = SEM)
Intake since last measure (g per mouse per day)
Group 1: Vehicle
Group 2: GA
Group 3: Avicin D (0.25mg/kg)
Group 4: Avicin D (0.5mg/kg)
Group 5: Avicin D (escalating dose to 2.0mg/kg)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
12/19/08
12/24/08
12/29/08
1/3/09
1/8/09
1/13/09
1/18/09
1/23/09
Date

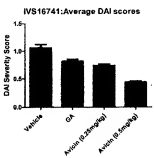

IVS16741: Cumulative Water Intake by Treatment Group
Intake Per Mouse (g)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
12/18/08
12/23/08
12/28/08
1/2/09
1/7/09
1/12/09
1/17/09
1/22/09
1/27/09
Date
Group 1: Vehicle
Group 2: GA
Group 3: Avicin D (0.25mg/kg)
Group 4: Avicin D (0.5mg/kg)
Group 5: Avicin D (escalating dose to 2.0mg/kg)

5.5 DAI Scoring System

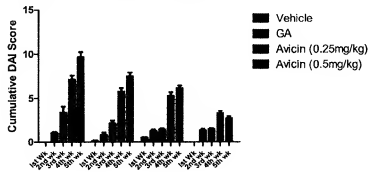
Disease Activity Index (DAI) scoring represents non-invasive observation of disease severity. Animals were examined individually, 3 times a week based on a scoring system given below. Observations were started during first DSS Cycle. No prolapse was seen in any of the groups. Fecal Occult blood was also not seen in any of the groups with the exception of few animals in group 5 during 3rd DSS cycle. DAI raw scores are embedded in Appendix 1.

Score	Prolapse	Stool Consistency	Fecal Occult Blood
0	Normal Anus	Solid Pellet	None
1	Partial	Semi-Solid	Occult Blood
2	Moderate	Soft Stool	Gross Blood
3	Full Prolapse	Diarrhea	-

Changes in stool consistency were noticed in different groups. Most animals in vehicle group showed diarrhea. Average DAI score was calculated for all groups and found to be higher in vehicle group. DAI scores representing stool consistency were higher in vehicle group during 4th DSS cycle. Cumulative scores calculated for each week of the study showed disease induction in all groups, most severe in vehicle group. All the groups showed increased disease activity during 4th week of observations. Disease activity scores continued to be higher during 5th week in all groups except for Avicin group (0.5mg/kg). This group showed less severity and disease induction as compared to other groups.



IVS16741: Cumulative DAI during study

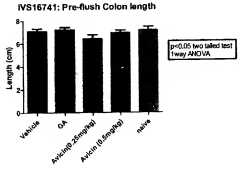


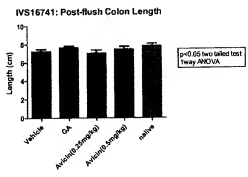
5.6 Colon Measurements

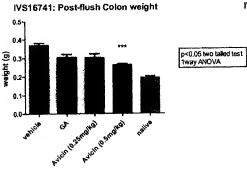
Mice were euthanized using inhaled carbon dioxide at the completion of the study and colons were harvested; measured, flushed, and weighed prior to fixation for histopathology.

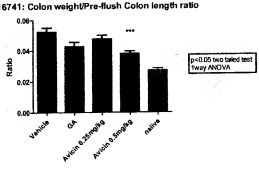
Colon lengths were compared before and after flushing the contents and didn't vary significantly between experimental groups. Post-flush colon weights were found to be significantly different in all groups. Vehicle group had significantly more weight as compared to high dose Avicin group ($p<0.05$ two-tailed t test)

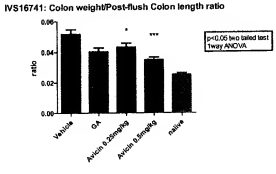
Colon weight/length ratio has been reported to be an indicator of colitis progression. Ratio was significantly higher in vehicle group as compared to Avicin high dose group (two tailed t test).











5.7 Histology

Formalin-fixed colons were sectioned and stained in Jax pathology laboratory. Disease severity scores were given using a scoring system given below. For each animal, distal,

proximal and medial (transverse) colon sections were scored for the following parameters. The three scored parameters (inflammation, glandular epithelial loss, erosion) were ultimately summed to arrive at a sum of histopathology scores, which indicated the overall damage and would have a maximum score of 15 for each colon segment.

Inflammation (foamy macrophages, lymphocyte and PMN infiltrate)

Normal = 0
Minimal = 1(generally focal affecting 1-10% of mucosa or if diffuse then minimal)
Mild = 2 (generally focal affecting 11- 25% of mucosa or if diffuse then mild)
Moderate = 3 (26-50% of mucosa affected with areas of gland loss replaced by inflammatory cell infiltrate, milder in remaining areas of mucosa)
Marked = 4 (51-75% of mucosa affected with areas of gland loss replaced by inflammatory cell infiltrate, milder in remaining areas of mucosa)
Severe = 5 (76-100% of mucosa affected with areas of gland loss replaced by inflammatory cell infiltrate, milder in remaining areas of mucosa)

Epithelial cell loss/damage scored individually using a % area involved scoring method:

None = 0
1-10% of the mucosa affected = 1
11-25% of the mucosa affected = 2
26-50% of the mucosa affected = 3
51-75% of the mucosa affected = 4
76-100% of the mucosa affected = 5

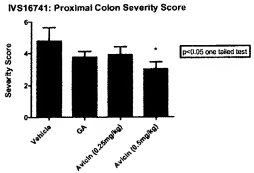
Parameters that were scored using % involvements include:
Colon glandular epithelial loss- this includes crypt epithelial as well as remaining gland epithelial loss
Colon erosion – this reflects loss of surface epithelium and generally is associated with mucosal hemorrhage

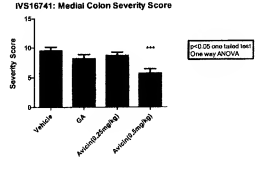
Inflammatory cell infiltrates in the colonic mucosa were evaluated for approximate % of neutrophils in the total infiltrate using the criteria below.

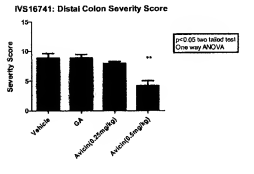
0 = approx 0%
10 = approx 10%
25 = approx 25%
50 = approx 50%
75 = 75% or greater

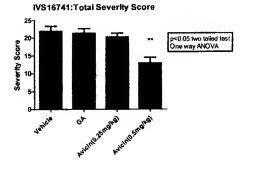
5.8 Histopathology Analysis

Histology findings and grading of individual slides was performed without knowledge of treatment (blinded). Once this was completed, pathologist was unblended to document histology summary. In all treatments groups, the medial and distal segments of the









colons received higher scores than the proximal and medial segments, consistent with previous DSS studies.

Group 4 of high dose Avicin group showed significantly low medial and distal disease severity as compared to vehicle group, low-dose Avicin group and positive control group of GA.

Total average scores were calculated for each group by adding proximal, medial and distal scores and are given below.

Group 1 Vehicle	21.9
Group 2 GA	21.2
Group 3 Avicin (0.25mg/kg)	20.2
Group 4 Avicin (0.5mg/kg)	12.8

Histological analysis showed that disease was induced in all the groups. Positive control Glatiramer Acetate group didn't show any significant improvement in disease outcome. Higher dose of Avicin (0.5mg/kg) showed significant low number of severe lesions as compared to all other three groups.

5.9 Analysis for toxicity in other tissues

Thirteen Samples of liver, kidney and skin from three experimental groups were analyzed for histological findings. Three animals from vehicle group and five animals each from Avicin low and high dose group were selected. Three tissues from each animal were formalin fixed and sectioned in the JAX pathology laboratory. Observations made by JAX histopathologist are recorded in a table embedded in Appendix 1. Photomicrographs taken to support these findings are copied on the attached CD.

5.10 Study Limitations and Deviations:

There were 13 unplanned mice deaths outside of the study protocol, allowing 74% of the subjects to be followed up.

Animals that Died on Study

Animal No.	Cause	Date
049 (Group 5)	FD	01/13/09
036 (Group 5)	EC	01/14/09
037 (Group 5)	EC	01/14/09
038 (Group 5)	EC	01/14/09
039(Group 5)	FD	01/14/09
040(Group 5)	EC	01/14/09
046 (Group 5)	EC	01/14/09
047 (Group 5)	EC	01/14/09
048(Group 5)	EC	01/14/09
050 (Group 5)	FD	01/14/09
007 (Group 1)	FD	01/19/09
019 (Group 2)	FD	01/19/09
034 (Group 2)	FD	01/29/09

FD = found dead
EC = euthanized for cause

The following inadvertent deviation occurred from the approved study protocol.

- On 12/10/08, due to a typo in the study plan, the first two mice in Group 2 (IVS16741016 and IVS16741017), were dosed at twice the intended volume of drug. The study plan indicated that mice were to be dosed at 0.01 x BW, but the mice were actually supposed to be dosed 0.005 x BW. The error was caught by the Study Director before any more mice were dosed incorrectly.